

The University of Chicago

STRUCTURAL AND METABOLIC
AFTER-EFFECTS OF SOAKING
SEEDS OF PHASEOLUS

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY
1932

By
WILLIAM M. BAILEY

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WILLIAM MARSHALL BAILEY

STRUCTURAL AND METABOLIC AFTER-EFFECTS OF SOAKING SEEDS OF PHASEOLUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 441

WILLIAM MARSHALL BAILEY

(WITH THREE FIGURES)

Introduction

The purpose of this investigation was to determine the influence which certain physiological conditions of the seed may exert upon subsequent development of the plant. The work was concerned mainly with the effects produced by soaking the seeds for a prolonged period in distilled water previous to planting them. KIDD and WEST first gave prominence to this problem by their experimental studies (4), and by an extensive review of the literature on related investigations (5-8). In this review were considered the effects of the conditions under which the parent plant had grown, the degree of ripeness, the conditions operating during germination and early seedling stages, and the effects of soaking the seeds in water and certain salt solutions in determining the subsequent course of development. The evidence in general seems to indicate that the factors which influence a plant during its earliest stages of development have a more or less pronounced effect upon its subsequent life history.

In their experiments, KIDD and WEST (4) soaked seeds under 4 cm. of distilled water at an average temperature of 17° C. for periods varying from 8 to 72 hours. Their report does not indicate that any provision was made for the elimination of bacteria or for aeration. The seeds used were *Phaseolus vulgaris* (dwarf bean), *Pisum sativum* (culinary pea), *Vicia faba* (broad bean), *Lupinus albus* (white lupine), *Helianthus annuus* (sunflower), *Triticum vulgare* (wheat), *Hordeum sativum* (barley), *Avena sativa* (oats), and *Brassica alba* (white mustard). The seeds were germinated on damp sand in porous flower pots covered with glass. The results showed that soaking the seeds more than 24 hours diminished the rate of germination in *Pha-*

seolus, *Pisum*, and *Helianthus*, and that there was a decrease in the rate of germination in *Hordeum* resulting from 72 hours of soaking.

They obtained data showing the effects of soaking seeds (dwarf bean, culinary pea, barley) on subsequent growth by growing them for 12 days on damp sand. They found that the soaking had a marked effect in retarding subsequent growth of the plants. They also carried on experimental work with seeds treated with distilled water and grown for longer periods in the greenhouse. Wheat, oats, white mustard, dwarf beans, broad beans, and white lupine were used. Samples of these seeds were soaked 6, 24, 48, and 72 hours respectively, and planted in potting soil in the greenhouse. In the case of dwarf bean harvested $4\frac{1}{2}$ weeks after sowing, a period of soaking of only 6 hours had a marked predetermining influence in retarding growth, which was greatly augmented by longer periods of treatment. Soaking for 3 days killed all of the seeds. Broad bean 3 weeks after planting showed diametrically opposite results, since there was a progressive increase in the height of the plants and in the rate of germination of the seeds resulting from the soaking, which was carried on up to 72 hours. Wheat showed a progressive increase in height and dry weight of tops in the plants from soaked seeds up to 48 hours of treatment. There was also a small increase in the rate of seed germination. However, plants from seeds soaked 72 hours were smaller and less vigorous. Oats showed results similar to those for wheat.

In addition, KIDD and WEST investigated the effects of temperature on the predetermining influence of soaking seeds of dwarf bean in distilled water (9). It was found that the temperature during the seed treatment had no marked effect upon the yield, but that the deleterious influence of soaking was somewhat less at temperatures near 20° C. than at temperatures higher or lower than this.

Three main conclusions were drawn from the results of this experimental work: first, that the soaking in distilled water previous to sowing may have a marked influence upon subsequent growth of the plants; second, that a germination test cannot be relied upon as a criterion of what this influence will be; third, that the nature of this influence is strongly specific. Thus very different results may be obtained from closely related plants. While soaking the seeds in water

greatly retarded the growth of the plants in the case of dwarf bean, this treatment increased both the rate of germination and the size of the plants produced in the case of broad bean.

TILFORD, ABLE, and HIBBARD (19), employing many kinds of seeds, found that deleterious effects are produced by prolonged water-soaking. Different results were secured from closely allied plants. A short period of soaking (8 hours) was found to interfere with germination of dwarf bean seeds and had a deleterious influence on subsequent development of the plants. The data submitted give results only on germination. In three tests sterilized seeds treated 72 hours in sterilized distilled water with aeration gave germination tests of 72, 100, and 88 per cent respectively. Non-sterilized seeds treated 72 hours in distilled water with aeration gave germination tests of 14, 40, and 27 per cent in three respective tests. Non-sterilized seeds soaked in distilled water 72 hours without aeration gave 20, 0, and 0 per cent of germination in three tests. It was concluded that the injurious influence on seed germination resulting from the soaking in distilled water is due to bacterial activity, and to a deficiency of oxygen and an excessive accumulation of carbon dioxide.

RHINE (15) found that soaking wheat for a period of 22 days in sterilized distilled water reduced the rate of germination to 2 per cent, while soaking for a period of 29 days resulted in a complete failure of germination.

BARTON (1), while experimenting on autolysis in seeds, investigated the influence of soaking for long periods of time on seed germination. Seeds of navy beans, peas, corn, and wheat were used. Four-ounce bottles, fitted with two-hole stoppers and right-angled glass tubes, were connected in series. The bottles and connections were sterilized. Each series of bottles was filled with boiling water. When at room temperature, the water was saturated with carbon dioxide. Five seeds, after being sterilized and washed with sterilized distilled water, were placed on glass wool in the first bottle of the series. After periods of soaking varying in length up to 3 months, the water was forced out with sterilized oxygen. In 3 or 4 days, 60 to 100 per cent of the seeds germinated. BARTON concludes: "(1) that the capacity for germination is not destroyed by long-continued soaking in sterile media; (2) that destructive changes are not brought

about by enzymes or ferments within the seeds; and (3) that any change that may take place within the seed does not in any way affect the viability and is not accompanied by any visible manifestation."

In the present investigation, the study of the predetermining influence of soaking seeds in distilled water has been carried further, in an effort to determine whether this influence is to be ascribed entirely to bacterial action and interference with respiration, or whether other causes are operating; and to discover some of the subsequent structural and metabolic effects of this seed treatment.

Experimentation

Early Valentine beans (*Phaseolus vulgaris*) were used in the investigation, seeds of relatively high germinating capacity being secured. The seeds were carefully sorted, only sound ones of approximately uniform size and regular shape being used.

The seeds were soaked in sterilized distilled water aerated by means of the apparatus shown in figure 1. In order to secure uniform movement of the air, it was drawn through the apparatus by means of an aspirator. The volume of air used was approximately 24 liters per day. It seems evident that lack of oxygen or the accumulation of carbon dioxide was not a limiting factor in this investigation, in view of the fact that TANG'S (18) results indicated that 6 liters of air per day was sufficient to give maximum results in the germination of wheat. The distilled water in flask *A* was sterilized in the autoclave, the flask being plugged with cotton, and cooled to room temperature. The cotton filters *F*₁ and *F*₂, carefully wrapped in heavy paper, were sterilized by prolonged heating in the autoclave. The remainder of the apparatus was sterilized by heating for 2 hours in steam at the temperature of boiling water, it being impossible to use the autoclave because of injury to the rubber connections by the high temperature. After sterilization the apparatus was connected up with as much precaution as possible to avoid contamination with bacteria and fungus spores from the air.

The seeds were presoaked 10 minutes in distilled water (2), after which they were put in the seed chamber *S*, which was then filled with a 0.25 per cent solution of "uspulun," to sterilize the seeds.

It was found in preliminary tests that this treatment in no way impaired germination. After 15 minutes the uspulun solution was drawn over into flask *E*, and replaced with water from flask *A* by means of suction from the aspirator. Approximately 1.5 liters of water was drawn through the seed chamber from flask *A* for the purpose of washing the seeds. Air was then drawn steadily through the seed chamber, after being sterilized by passing through the cotton

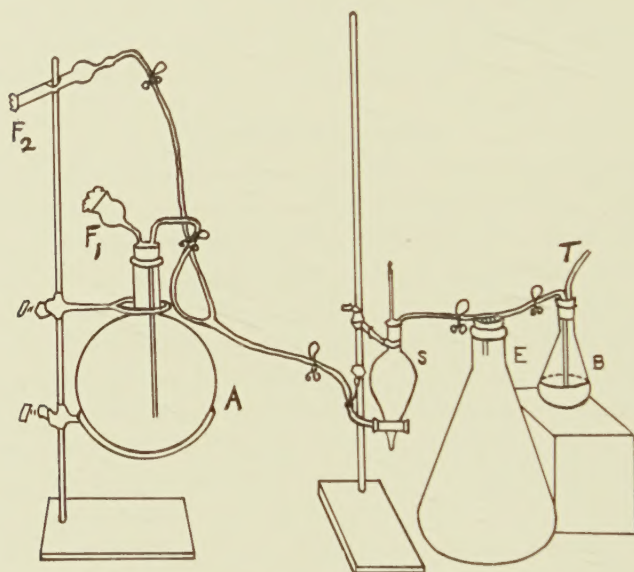


FIG. 1.—Apparatus for soaking seed with aeration: *S*, seed chamber; *A*, flask containing sterilized distilled water; *E*, large flask into which water was drawn from seed chamber; *F*₁, *F*₂, cotton filters; *B*, safety flask; *T*, tube connected to aspirator.

filter *F*₂. The water was changed four times per day by being drawn from flask *A* into the seed chamber *S* and into flask *E* by suction from the aspirator. When the water was all drawn from flask *A* it was replaced from time to time with flasks similarly filled with distilled water, sterilized and cooled, as much precaution as possible being taken to prevent contamination from the air in changing the flasks.

To test the success in the elimination of bacteria and fungi, a quantity of nutrient broth was put into a flask of the same size as

flask *E* and plugged with cotton. The flask with its contents was sterilized in the autoclave, cooled, and then connected with the apparatus in the place of flask *E*, as much precaution as possible being taken to avoid contamination from the air. A small quantity of the water in which the seeds had been soaking was drawn from the seed chamber into the broth. After incubation in a warm room for a period of 3 days, no indication of bacteria or fungi appeared in the broth. This test was made during the third day of the seed treatment.

In each experiment in which the seeds were planted, the seeds used for control were sterilized in the usual way, then washed as quickly as possible and planted at once. They are called "untreated seeds" in the report of the results.

The plants were grown in the greenhouse and were watered with the usual water supply available there. The temperature of the greenhouse usually varied between 60° and 80° F. The seeds were planted at a depth of approximately 1 inch. The plants were harvested when they had reached the blooming stage, when they were considered mature. The tops were harvested, the stems being cut at the surface of the soil.

EXPERIMENT I

The purpose of this experiment was to determine the effect of the seed treatment on germination, total number of mature plants produced, growth, average weight, time required to reach maturity, and influence of seed treatment on stem and leaf structure. The temperature at which the seeds were treated was 16°–19° C. The following seed treatments were used: no. 1, seeds untreated; no. 2, seeds treated 8 hours; no. 3, seeds treated 2 days; no. 4, seeds treated 5 days; no. 5, seeds treated 7 days; no. 6, seeds treated 9 days. This experiment was carried out between March 22 and May 9, 1930.

STEM AND LEAF STRUCTURE.—Cross-sections of the stems in the internode just above the first pair of leaves were prepared from the mature plants. The average diameter of the stem and the average thickness of the xylem and phloem in this internode were determined in the plants from the untreated seeds and in those from seeds treated 5 days. Also the ratio of the average thickness of the phloem

to that of the xylem was determined. Cross-sections of leaves from the mature plants of these two sets were also prepared. For the sake of uniformity, the leaf sections were all taken from the terminal leaflet of the uppermost mature leaf. The leaf structures of the two sets of plants were compared.

EXPERIMENT II

This experiment attempted the determination of the influence of the seed treatment on the percentage of water and dry matter in the plants produced, the abundance of various carbohydrates and nitrogen compounds, and the influence on the growth of the plants of a calcium salt in very dilute solution in the water in which the seeds were soaked. The experiment ran from November 22, 1930 to January 20, 1931. The following were the respective seed treatments: no. 1, seeds untreated; no. 2, seeds treated in sterilized distilled water with aeration 5 days; no. 3, seeds treated in sterilized distilled water containing calcium nitrate (approximately 900×10^{-6} gm. normal per liter) with aeration 5 days. The temperature was 15° – 16° C. The plants were grown in the greenhouse and harvested at maturity. The three sets of plants were harvested at the same hour of the day, 8 A.M.

Analyses for determining the percentage of dry matter, carbohydrates, and nitrogen compounds were made on samples of plants from no. 1 and no. 2. Determinations were made in duplicate. All analyses of the extractives were made within 3 weeks after harvesting. The Methods of Analysis of the A.O.A.C. (13) were generally followed in the chemical determinations.

EXTRACTION. Not less than 20 plants (14) were ground together in a Russwin mill, and the ground material thoroughly mixed. Samples of this material were weighed off into sufficient 95 per cent alcohol, boiling hot, to make the alcohol content of the entire mass approximately 85 per cent. To this material was added 0.5 gm. of pure calcium carbonate to neutralize the acids present and thus prevent hydrolysis. The samples, in large pyrex beakers covered with watch glasses, were kept heated to the boiling temperature of the alcohol on the hot water bath for 40 minutes. The samples were then extracted in Soxhlet extractors with 95 per cent alcohol, ether, and

water, each for 2-4 hours, then with 95 per cent alcohol over night. All alcohol used for preserving and extracting the samples was redistilled in glass, and only chemically pure ether was used. The alcohol, ether, and water used in the extraction were poured together into a large evaporating dish, and the alcohol and ether were replaced with water by evaporation. The extract was then transferred quantitatively to a volumetric flask and the volume adjusted by the addition of water. The lipoid materials, together with the chlorophyll, which were precipitated out by the replacement of the alcohol and ether with water, were removed in solution in 20 cc. of chloroform. The chloroform solution was then evaporated to dryness and the weight of the substances dissolved in the chloroform determined. Since this material consists mainly of chlorophyll and the carotinoid pigments, and very little difference was found between the percentages in the plants from treated seeds and those from untreated seeds, it was assumed that there was no important difference in the quantity of these pigments in the two sets of plants.

TOTAL WEIGHT OF SOLIDS.—The total dry weight of the solids was determined by taking the sum of the dry weight of the solid residue left after extraction, the solids of the extract computed from an aliquot evaporated to dryness and weighed, and the dry weight of the substances removed from the extract in solution in chloroform.

SUGARS.—In estimating reducing sugars, 50 cc. of the extract was measured off, cleared with basic lead acetate solution, and delead with potassium oxalate solution. The volume was then adjusted, and the reducing sugars of 50 cc. were computed as glucose by the Bertrand volumetric method, and calculated for the entire sample.

The non-reducing sugars were computed as sucrose. From the solution previously cleared and delead, 50 cc. was measured off and 5 cc. of HCl added. This mixture was heated for 1 hour on the hot-water bath and then set aside for 48 hours. It was then neutralized with sodium hydroxide and sodium carbonate, and the volume adjusted to 100 cc. The total reducing sugar was computed as invert sugar by the Bertrand volumetric method. The reducing-sugar content before hydrolysis was also computed as invert sugar. The difference between these multiplied by 0.95 gave the sucrose, which was computed for the entire sample.

POLYSACCHARIDES. For the determination of the total polysaccharides, a small sample of the dry solid residue was weighed off into a Kjeldahl flask, and to this were added 200 cc. of water and 12.5 cc. of concentrated HCl. This mixture was boiled under a reflux condenser for 3 hours. The mixture was then cooled and neutralized with sodium hydroxide and sodium carbonate, cleared and deleded, and its volume adjusted. The reducing sugar was estimated as glucose by the Bertrand method, and the weight of the polysaccharides was calculated by multiplying this result by 0.9. The weight of the polysaccharides was then calculated for the entire sample.

The starch was estimated from the results of saliva digestion, the method employed by CLEMENTS (3) in determining the starch content of leaves. To secure gelatinization of the starch, a small amount of the dry residue left after extraction was weighed off, put in a pyrex beaker, and moistened with water. After a few minutes 50 cc. of water was added. The mixture was then boiled for a moment over a gas burner, the inside of the beaker washed down, and the beaker, covered with a watch glass, transferred to the hot-water bath for 1 hour. The contents of the beaker were then cooled to approximately 37° C., and 10 cc. of filtered saliva was added. The mixture was incubated over night at 37° C. A check sample of the material was treated in the same way, and samples of this were tested with iodine and examined microscopically to make sure that the hydrolysis of the starch was complete.

After the starch digestion was complete, the mixture was cleared, deleded, and its volume adjusted. From this, 100 cc. was taken and acidified to the extent of 2.5 per cent with sulphuric acid and heated on a boiling hot-water bath for 1.5 hours to hydrolyze to glucose the maltose resulting from the starch digestion. The solution was then neutralized with sodium hydroxide and sodium carbonate and its volume adjusted. The glucose was estimated by the Bertrand method and the starch calculated by multiplying this result by 0.9.

The hemicelluloses were determined by taking the difference between the total polysaccharides and the starch.

TOTAL CARBOHYDRATES. The total carbohydrate content was determined by adding together the reducing sugars, sucrose, and total polysaccharides.

NITROGEN COMPOUNDS.—The total nitrogen of the extract was determined by the Gunning method modified to include the nitrogen of the nitrates. The organic nitrogen of the extract was determined by the Gunning method. For these determinations 50 cc. samples of the extract were dried in Kjeldahl flasks on the hot-water bath. The amino-acid nitrogen of the extract was determined by the Van Slyke method. The nitrogen of the solid residue was determined by the Gunning method. The weight of the protein was obtained by multiplying this result by 6.25. The total nitrogen of the sample was found by taking the sum of the total nitrogen of the extract and that of the solid residue.

EXPERIMENT III

In this experiment the respiratory carbon dioxide was determined by the method of double titration, and the rates of respiration in the treated and untreated seeds after sprouting were thus compared. The air was drawn into the respiratory chamber through a series of five wash bottles, the first four containing sodium hydroxide solution and the last barium hydroxide; and from the respiratory chamber through the gas absorption bottle (Milligan) containing 250 cc. of normal sodium hydroxide solution for the absorption of the respiratory carbon dioxide; then through a wash bottle containing barium hydroxide, and a safety flask connected with the aspirator by means of which the air was drawn through the apparatus at a uniform rate.

Two sets of seeds were used. In set no. 1 the seeds were untreated (presoaked, sterilized, and washed); in set no. 2 the seeds were presoaked, sterilized, washed, and soaked in sterilized distilled water with aeration in the usual way 3 days. The seeds of each set were sprouted in a germinator until the sprouts were approximately 2 cm. long before being put in the respiration chamber. Many of the seeds of set no. 2 did not sprout. Twenty-five sprouted seeds were used in each of the tests. These respiration tests were carried out at a temperature of approximately 25° C. They were made in duplicate, and were continued exactly 10 hours. At the end of this period the solution in the gas absorption bottle was titrated with normal and 0.1 normal sulphuric acid with phenolphthalein indicator, and then with 0.1 normal sulphuric acid with methyl orange indicator. After correction with blank tests on the normal sodium hydroxide, the second

titration gave the measurement of the respiratory carbon dioxide. As a result of the first titration, the carbon dioxide is held in the form of NaHCO_3 . The carbon dioxide is set free in the second titration.

EXPERIMENT IV

In this experiment the effect on catalase activity of soaking seeds in distilled water both with and without aeration was investigated. For washing and soaking the seeds, distilled water sterilized in the autoclave and cooled in the ice-box was used. The seed treatment consisted of presoaking in distilled water 10 minutes, sterilizing with 0.25 per cent uspulun solution 15 minutes, washing with distilled water, and soaking in distilled water. After soaking the seeds were dried in the draft of an electric fan for 48 hours to the air-dry state (12). The untreated seeds were used in their original dry state, but were exposed to the draft of the electric fan with the soaked seeds in order to bring them as nearly as possible to the same degree of dryness.

The method employed by SHULL and DAVIS (17) was followed in the catalase tests, Oakland "dioxogen" being used. The tests were run in triplicate. Not less than 20 seeds were used in preparing the material for each test. The seed coats, hypocotyls, and plumules were removed. The cotyledons were finely ground in a glass mortar, and this material was sifted through a 100-mesh sieve. Of this material, 0.2 gm. was used in each test. In order to neutralize acids, 0.5 gm. of pure calcium carbonate was mixed with 10 cc. of the dioxogen in the funnel of the catalase apparatus. The percentage of dry matter in the pulverized material was determined, and the oxygen liberated by the catalase was calculated on the basis of the dry weight.

In experiment IVa, three sets of seeds were used. In set no. 1 the seeds were untreated; in sets 2 and 3 the soaking with aeration was continued 2 and 5 days respectively, the water being changed four times daily during the soaking. Each seed chamber was placed in a water bath and the temperature thus kept at 12° – 14° C. The apparatus and method employed in soaking the seeds with aeration were those used in the preceding experiments (fig. 1). In experiment IVb also three sets of seeds were used. In set no. 1 the seeds were untreated; in sets 2 and 3 the soaking without aeration was con-

tinued 2 and 5 days respectively. The seeds were soaked in Erlenmeyer flasks of 500 cc. capacity, plugged with cotton, and previously sterilized in the autoclave. Precautions were taken to prevent contamination of the seeds with bacteria, and no indications of bacterial growth during soaking were seen. The water was not changed during the period of soaking. The seeds were kept in the ice-box at a temperature of 12°–14° C. during the soaking period.

Results

EXPERIMENT I.—The results of this experiment show a progressive decrease in the rate of germination resulting from the seed treatment. There is a still greater reduction in the number of mature plants pro-

TABLE I

INFLUENCE OF SEED TREATMENT ON GERMINATION (APPEARANCE OF SEEDLINGS AT SURFACE OF SOIL), NUMBER OF MATURE PLANTS PRODUCED, AVERAGE WEIGHT, AND NUMBER OF DAYS REQUIRED TO REACH MATURITY

PERIOD OF TREATMENT	NO. OF SEEDS	NO. GERMINATED	PERCENT-AGE GERMINATION	NO. OF MATURE PLANTS	PERCENT-AGE MATURED	AV. WEIGHT OF PLANTS (GM.)	DAYS TO REACH MATURITY
Untreated.....	100	95	95.00	91	91.00	26.26	37
8 hours.....	120	97	80.83	84	70.00	24.50	38
2 days.....	120	76	63.33	71	59.17	22.88	38
5 days.....	140	46	32.86	31	22.14	17.48	43
7 days.....	140	7	5.00	3	2.14	16.67	48
9 days.....	140	1	0.71	1	0.71	14.50	46

duced, since a considerable number of them from treated seeds were unable to survive until they reached maturity. There is also a progressive decrease in the average weight of the tops, and an increase in the time required for the plants to reach maturity with the lengthening of the period of seed treatment. The results are shown in table I.

Figure 2 shows the effect of the seed treatment on growth of the plants. The two plants were at the same stage of development. They were grown in flower pots, and the roots were removed from the soil so as to secure the entire root system. The plant on the left is typical of those grown from untreated seed, that on the right of those grown from seed treated 5 days. Flower buds may be seen on each of them. The plant from untreated seed reached this stage in

44 days, that from treated seed in 50 days. The plants from treated seeds showed marked reduction in growth resulting from the seed treatment. Both the shoot and the root system showed this reduction in growth, apparently in about the same proportion.



FIG. 2.—Effects on subsequent growth of soaking bean seeds in distilled water with aeration: left, plant from untreated seed, 44 days after planting; right, plant from seed soaked with aeration 5 days, 50 days after planting.

In the plants from untreated seeds the average diameter of the internode of the stem immediately above the first pair of leaves was 3.497 mm., the average thickness of the xylem was 0.474 mm., and the average thickness of the phloem was 0.07953 mm. The ratio of the average thickness of the phloem to the xylem was 0.168. In the

plants produced from seeds treated 5 days the average diameter of the same internode of the stem was 3.377 mm., the average thickness of the xylem 0.452 mm., and the average thickness of the phloem 0.1155 mm. The ratio of the phloem to the xylem was 0.255. It will be noted that there is a marked increase in the average thickness of the phloem and in the ratio of the phloem to the xylem in the plants from the treated seeds.

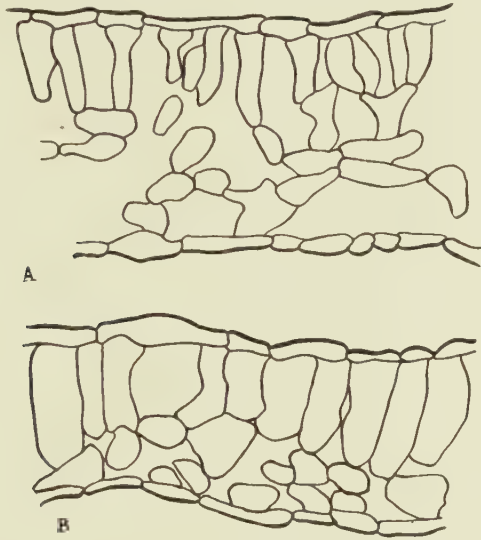


FIG. 3.—Cross-sections of leaves: *A*, section of typical leaf of plant from untreated seed; *B*, section of typical leaf of plant from seed soaked with aeration 5 days.

The difference in leaf structure in the plants from untreated seeds and those from seeds treated 5 days is shown in figure 3. Figure 3 *A* shows the cross-section of a leaf of a mature plant from untreated seed, figure 3 *B* shows that of a mature plant from seed treated 5 days. The sections shown in the figure are representative of the leaf structure found generally in the two sets of plants, the sections being drawn to the same scale. There is a marked decrease in thickness and increase in compactness of structure in the leaf of the plant from the treated seed.

EXPERIMENT II.—This experiment deals with the influence of soaking seeds in distilled water with aeration on germination, num-

ber of mature plants produced, average weight, time required to reach maturity, percentage of dry matter and water content, abundance of carbohydrates and nitrogenous compounds; and the influence on growth of the presence of a calcium salt in very dilute solution in the water in which the seeds were soaked.

Because of the shorter days and lower light intensity of late autumn and winter, there was but little branching in these plants, and their size and weight were much less than in the plants in experiment I. The plants grew and matured normally, however, and the results

TABLE II

GERMINATION OF SEEDS (APPEARANCE AT SURFACE OF SOIL), NUMBER OF MATURE PLANTS PRODUCED, AVERAGE WEIGHT, AND NUMBER OF DAYS REQUIRED TO REACH MATURITY:

1, SEEDS UNTREATED

2, SEEDS SOAKED WITH AERATION 5 DAYS IN DISTILLED WATER

3, SEEDS SOAKED WITH AERATION 5 DAYS IN DISTILLED WATER CONTAINING CALCIUM NITRATE (APPROXIMATELY 900×10^{-6} GM. NORMAL PER LITER)

No.	NO. OF SEEDS	NO. GERMINATED	PERCENT- AGE GER- MINATION	NO. OF MATURE PLANTS	PERCENT- AGE MATURED	AV. WEIGHT OF PLANTS (GM.)	DAYS TO REACH MATURITY
1.....	100	90	90.00	80	80.00	2.80	41
2.....	140	98	70.00	54	38.57	1.86	48
3.....	105	71	67.62	48	45.71	2.08	48

indicate similar effects from seed soaking in reduction of growth. There is a decrease in germination and a greater decrease in the number of plants able to reach maturity, a marked reduction in average weight, and an increase in the time required to reach maturity. The results obtained from the addition of calcium nitrate to the water indicate that in general this addition did not greatly modify the effects of the soaking. The results are shown in tables II and III.

The data given in table III indicate that there is an increase in the percentage of dry matter in the plants produced from seeds soaked with aeration 5 days in distilled water, in comparison with the plants from untreated seeds.

One of the most striking results of the predetermining influence of soaking the seeds is the relative increase in the carbohydrates in

the plants produced from the treated seeds, in comparison with those from untreated seeds. This increase is seen in the reducing

TABLE III
PERCENTAGE OF DRY MATTER AND MOISTURE

SEED TREATMENT	PERCENTAGE	
	DRY MATTER	MOISTURE
Untreated.....	16.61	83.39
5 days in H ₂ O.....	19.05	80.95

TABLE IV
GLUCOSE, SUCROSE, AND TOTAL SUGARS EXPRESSED AS PERCENTAGES
ON TOTAL-WEIGHT AND DRY-WEIGHT BASES

SEED TREATMENT	WEIGHT BASIS					
	GLUCOSE		SUCROSE		TOTAL SUGARS	
	TOTAL	DRY	TOTAL	DRY	TOTAL	DRY
Untreated.....	0.299	1.800	0.309	1.921	0.608	3.721
5 days in H ₂ O.....	0.443	2.320	0.344	1.804	0.787	4.124

TABLE V
STARCH, HEMICELLULOSES, AND TOTAL POLYSACCHARIDES EXPRESSED AS
PERCENTAGES ON TOTAL-WEIGHT AND DRY-WEIGHT BASES

SEED TREATMENT	WEIGHT BASIS					
	STARCH		HEMICELLULOSES		TOTAL POLYSACCHARIDES	
	TOTAL	DRY	TOTAL	DRY	TOTAL	DRY
Untreated.....	0.672	3.857	1.212	7.481	1.883	11.338
5 days in H ₂ O.....	1.635	8.587	1.330	6.985	2.965	15.572

sugars and total sugars, starch, and total polysaccharides. These results are shown in tables IV–VII. The reducing sugars are computed as glucose and the non-reducing sugars as sucrose.

The data given in tables VIII and IX indicate that the predetermining influence of soaking the seeds resulted in a decrease in total nitrogen, organic nitrogen, amino-acid nitrogen, and proteins.

TABLE VI
TOTAL CARBOHYDRATES EXPRESSED AS PERCENTAGES
ON TOTAL-WEIGHT AND DRY-WEIGHT BASES

SEED TREATMENT	WEIGHT BASIS	
	TOTAL	DRY
Untreated.....	2.491	15.059
5 days in H ₂ O.....	3.752	19.699

TABLE VII
CARBOHYDRATES EXPRESSED AS PERCENTAGES OF WEIGHTS OF THE CARBOHYDRATES
FOUND IN PLANTS FROM UNTREATED SEEDS; BASIS OF EQUAL WEIGHTS OF
WET AND DRY MATTER OF SAMPLES

SEED TREATMENT	WEIGHT BASIS											
	GLUCOSE		SUCROSE		TOTAL SUGARS		STARCH		HEMICEL- LULOSES		TOTAL CARBO- HYDRATES	
	WET	DRY	WET	DRY	WET	DRY	WET	DRY	WET	DRY	WET	DRY
None.....	100	100	100	100	100	100	100	100	100	100	100	100
5 days in H ₂ O.....	148	129	111	94	129	111	243	223	110	93	151	131

TABLE VIII
TOTAL NITROGEN, ORGANIC NITROGEN, AMINO-ACID NITROGEN, AND PROTEINS
EXPRESSED AS PERCENTAGES OF WEIGHTS OF WET
AND DRY MATTER OF SAMPLES

SEED TREATMENT	WEIGHT BASIS							
	TOTAL NITROGEN		ORGANIC NITROGEN		AMINO-ACID NITROGEN		PROTEINS	
	WET	DRY	WET	DRY	WET	DRY	WET	DRY
None.....	0.623	3.749	0.619	3.729	0.021	0.126	2.745	16.531
5 days in H ₂ O...	0.543	2.851	0.540	2.836	0.017	0.088	2.545	13.303

EXPERIMENT III. This experiment, designed to measure the effects of seed soaking upon subsequent respiration, presents a quantitative determination of the carbon dioxide given off in respiration by treated and untreated seeds after sprouting. As is shown by table X, there was no decrease in respiratory carbon dioxide from sprouted seeds in the case of the soaked seeds in comparison with those not soaked. On the contrary, the results showed an increase.

TABLE IX

TOTAL NITROGEN, ORGANIC NITROGEN, AMINO-ACID NITROGEN, AND PROTEINS EXPRESSED AS PERCENTAGES OF WEIGHTS OF THESE FOUND IN PLANTS FROM UNTREATED SEEDS; BASIS OF EQUAL WEIGHTS OF WET AND DRY MATTER OF SAMPLES

SEED TREATMENT	WEIGHT BASIS							
	TOTAL NITROGEN		ORGANIC NITROGEN		AMINO-ACID NITROGEN		PROTEINS	
	WET	DRY	WET	DRY	WET	DRY	WET	DRY
None.....	100	100	100	100	100	100	100	100
5 days in H ₂ O...	87	76	87	76	80	70	93	81

TABLE X

WEIGHT OF RESPIRATORY CARBON DIOXIDE GIVEN OFF PER 100 GM. OF DRY MATTER IN 10 HOURS

	SEED TREATMENT	
	UNTREATED	3 DAYS IN H ₂ O
Grams of CO ₂	1.191	1.568

EXPERIMENT IV.—It was thought that soaking the seeds might influence their catalase activity. The data given in table XI indicate that in the case of seeds soaked with aeration there is a small decrease in catalase activity in the seeds soaked 2 days, but an increase in those soaked 5 days. On the other hand, table XII shows that in the case of the seeds soaked without aeration there is a considerable decrease in catalase activity resulting from soaking for 2 days, and a further decrease from a soaking period of 5 days. The

volume of oxygen has been computed for 1 gm. of air-dry material and 1 gm. of dry matter.

TABLE XI

VOLUME OF OXYGEN LIBERATED BY CATALASE ACTION AND PERCENTAGE OF OXYGEN ON BASIS OF THAT LIBERATED BY UNTREATED SEEDS; SEEDS SOAKED WITH AERATION; VOLUMES CORRECTED TO 0° C. AND 760 MM. PRESSURE; 10-MINUTE RUNS

SEED TREATMENT	0.2 GM AIR- DRY MATERIAL (cc.)	1.0 GM AIR- DRY MATERIAL (cc.)	1.0 GM. DRY MATTER (cc.)	PERCENTAGE OXYGEN
Untreated.....	19.97	99.85	113.04	100.00
2 days in H ₂ O...	19.04	95.20	108.02	95.56
5 days in H ₂ O...	22.49	112.45	129.47	114.53

TABLE XII

VOLUME OF OXYGEN LIBERATED BY CATALASE ACTION AND PERCENTAGE OXYGEN ON BASIS OF THAT LIBERATED BY UNTREATED SEEDS; SEEDS SOAKED WITHOUT AERATION; VOLUMES CORRECTED TO 0° C. AND 760 MM. PRESSURE; 10-MINUTE RUNS

SEED TREATMENT	0.2 GM. AIR- DRY MATERIAL (cc.)	1.0 GM. AIR- DRY MATERIAL (cc.)	1.0 GM. DRY MATTER (cc.)	PERCENTAGE OXYGEN
Untreated.....	23.02	115.10	125.89	100.00
2 days in H ₂ O...	20.32	101.60	111.87	88.86
5 days in H ₂ O...	19.27	96.35	106.43	84.54

Discussion

KIDD and WEST (4) found no decrease in the rate of germination in bean seeds soaked 6 hours, and but little decrease from a treatment of 24 hours; but practically complete failure of germination resulting from treatment for 72 hours. Their report shows no provision for aeration of the seeds or elimination of bacteria. TILFORD, ABLE, and HIBBARD (19), who sterilized the seeds to eliminate bacteria and aerated them during the soaking treatment, came to the conclusion that bean seeds may be expected to germinate as well under water as in the soil, provided there are sufficient oxygen for respiration, removal of the carbon dioxide and other by-products, and elimination of the bacteria. RHINE (15) found that wheat soaked in sterilized distilled water 22 days gave a germination test of only 2

per cent, while that soaked 29 days showed a complete failure of germination. BARTON (1) concluded that "the capacity for germination is not destroyed by long continued soaking in sterile media."

In this investigation, the seeds after soaking were planted at a depth of approximately 1 inch in the soil. Germination was considered to have taken place when the seedlings appeared at the surface of the soil. The results of the germination tests are shown in table I. It will be noted that soaking for 8 hours decreased the rate of germination from 95 to 80.83 per cent, and that there was a progressive decrease in the rate of germination as the length of the period of seed treatment was increased. The rate of germination was reduced to 5 per cent by 7 days' treatment. It should be borne in mind that in these experiments the seeds were freed from bacteria by sterilization, and that they were aerated during the period of soaking. The seeds were subjected to the action of a large volume of water, since the water used in soaking was completely changed four times per day. It is evident that soaking in distilled water had an injurious effect on germination, and that germination practically ceased when the seeds had been subjected to soaking for 7-9 days. These results seem to differ radically from those obtained by KIDD and WEST, TILFORD, ABLE, and HIBBARD, and BARTON. In the work of these investigators, the seeds were subjected to the action of comparatively small volumes of water, since the water was not changed during the soaking period. Also these workers did not germinate the seeds in the soil, while in this investigation the seeds were planted in the soil and the seedlings had to grow sufficiently to appear at the surface of the soil before the seeds were considered to have germinated. Table II shows the injurious effect on germination of a prolonged period of soaking. Data given in tables I and II show the further reduction in the number of plants from soaked seeds which were able to reach maturity, a considerable number of plants from treated seed dying before the mature stage was reached.

The plants photographed in figure 2 show the deleterious influence of seed soaking on subsequent growth of the plant. The reduction in growth in the shoot and root system is in about the same proportion. The increase in time required for the plants from treated seeds to reach the blooming stage also indicates the injurious effect of the

seed treatment on the rate of growth. This is further shown in the progressive decrease in the average weight of the plants (tables I, II).

TRUE (20) studied the harmful action of distilled water and the significance of calcium for higher green plants, using the electrical conductivity method. He found that the samples of distilled water showing the highest electrical resistance were in general more harmful to lupine roots than water containing larger quantities of electrolytes. He also found that these samples of water withdrew electrolytes from the tissues of the roots. This was taken to be the probable mechanism causing the harmful action. According to TRUE, the distilled water seems to withdraw material required for maintenance of the efficient action of the protoplasmic limiting membranes, with the result that cell permeability is increased and a further dissociation of the electrolytes from their points of combination in the proteins and other chemical structures of the cells ensues; but when a calcium salt is added to the distilled water in sufficient concentration to make it osmotically equal to tap water, this dissociating power of the water is largely undeveloped, the leaching is checked, and the chemical integrity of the cells is maintained. TRUE and BARTLETT (21) came to the conclusion that there are equilibrium concentrations for calcium and nitrate ions and for magnesium and nitrate ions at which leaching is largely overcome, and below which roots would leach ions into either solution or into both solutions mixed in various proportions. It was shown that calcium ions differ from magnesium ions in being harmless in concentrations that prove fatal in the case of magnesium. In no concentration of a calcium salt that was tried, the strongest being approximately 900×10^{-6} gm. normal per liter, was there any indication of injury.

SCARTH (16) investigated the toxic action of distilled water on *Spirogyra* and its antagonism by cations. He came to the conclusion that distilled water free from metal ions but exposed to the air is highly toxic to this plant, that the positive toxic factor is the H-ion concentration due to the dissolved CO_2 , and that cations are antagonistic to this toxic action according to their valencies, being limited also by their own toxicity.

In this investigation, experiment II was modified to determine

whether the addition of a calcium salt to the distilled water in which the seeds were soaked would decrease or modify the deleterious influence of soaking on subsequent growth of the plants. The highest concentration of calcium nitrate employed by TRUE, approximately 900×10^{-6} gm. normal per liter, was used. The data in table II lead to the conclusion that the injurious effects of soaking on the rate of germination and on subsequent growth were not greatly modified by the addition of the calcium salt. Table III shows that one of the results of soaking is an increase of dry matter or decrease in moisture in the mature plants produced, in comparison with plants produced from untreated seeds.

One of the very evident effects of the predetermining influence of seed soaking is the higher percentage of carbohydrates in the mature plants from the treated seeds. This is shown in tables IV–VII. The percentage of reducing sugar is higher in the plants from the treated seeds, and the increase in starch resulting from the seed treatment is very striking.

The data in tables VIII and IX lead to the conclusion that another effect of the predetermining influence of seed soaking is a relative decrease in total nitrogen, organic nitrogen, amino-acid nitrogen, and proteins. While this decrease in nitrogen compounds is not so pronounced as the increase in carbohydrates, yet it seems to be very evident.

A correlation between the predetermining influence of this seed treatment and the internal metabolism of the plants produced from the treated seeds may be seen in the carbohydrate-nitrogen relations of the plants, and the moisture and dry matter content. The plants from treated seeds, which were higher in carbohydrates and lower in nitrogen and nitrogen compounds, were lower in moisture content and higher in dry matter. On the other hand, the plants from untreated seeds, which were lower in carbohydrates and higher in nitrogen and nitrogen compounds, were higher in moisture and lower in dry matter (10).

It seems probable that the greater accumulation of carbohydrates in the plants from the treated seeds is due to a decrease in the ability of these plants to form amino acids, proteins, and related nitrogen compounds from the carbohydrates, resulting from soaking the

seeds. The reduction in growth in these plants may be a result of a reduction in the nitrogen compounds. A comparison of the two plants shown in figure 2 leads to the conclusion that the plant from the treated seed has as extensive a root system in proportion to the shoot as has that from the untreated seed. Evidently the decrease in the nitrogen compounds and water in the plants from the treated seeds is not due to reduced absorption caused by a poorly developed root system.

The data in table X indicate that there was no decrease in the production of respiratory carbon dioxide by the sprouted seeds resulting from soaking. Some of the soaked seeds did not sprout, of course, and only sprouted seeds were used in the test. This result indicates that the soaking treatment does not injure the respiratory enzymes or mechanism of the seeds, and that the reduction in growth is not due to a decrease in the rate of respiration. In fact, the production of respiratory carbon dioxide was somewhat higher in the sprouted seeds previously soaked than in those not previously soaked. Probably the higher water content of the former was more favorable to the respiratory process. The water content of the sprouted seeds previously soaked, at the stage at which the test for respiratory carbon dioxide was made, was 63.9 per cent; while that of the seeds sprouted without being previously soaked, at the same stage, was 53.7 per cent. MAXIMOV (11) gives the results obtained by KOLKWITZ in connection with the relation of moisture to respiration in barley. He found that 1 kg. of barley grain containing 10-12 per cent of moisture exhaled in 24 hours 0.0003-0.0004 gm. of carbon dioxide; with 14-15 per cent moisture, 0.0013-0.0015 gm. of carbon dioxide; and that with a further increase in water content, respiration increased rapidly, reaching 2.0 gm. of carbon dioxide in 24 hours, when the grain was almost completely swollen.

Table XI indicates that soaking 2 days in distilled water with aeration caused a decrease of 4.44 per cent in the oxygen liberated from hydrogen peroxide by the catalase activity of the seeds, while soaking with aeration 5 days caused an increase of 14.53 per cent. On the other hand, the data in table XII show that soaking without aeration resulted in a continuous decrease in catalase action, amounting to 11.14 per cent in the seeds soaked 2 days and 15.46 per cent in

those soaked 5 days. The latter result is in harmony with that obtained by RHINE (15). In using wheat soaked (without aeration) in distilled water previously boiled and cooled, she found that there was a continuous decrease in catalase activity resulting from soaking, the catalase action practically ceasing after a soaking period of 29 days. In the case of soaking with aeration, the seeds passed through the early stages of germination during the soaking treatment. There was the usual development of catalase activity in connection with germination, amounting to an increase of 14.53 per cent when the seeds had been soaked 5 days.

Summary

1. Soaking seeds of dwarf bean in distilled water with aeration resulted in a considerable decrease in the rate of germination (appearance of the seedlings at the surface of the soil) for periods of soaking as short as 8 hours. Practically a complete failure of germination resulted from soaking periods of 7 to 9 days.

2. The soaking treatment resulted in a still further reduction in the number of mature plants produced, as some of the seedlings from the treated seeds were unable to reach maturity.

3. The soaking treatment resulted in a progressive decrease in growth and weight of the plants produced and an increase in the time required for the plants to reach maturity.

4. The seed treatment resulted in general in a modification of the leaf structure, the leaf blades of plants from treated seeds generally being thinner and more compact in structure than those of plants from untreated seeds.

5. The greater average thickness of the phloem and higher ratio of the phloem to the xylem in the stems of the plants from soaked seeds is probably correlated with the relatively higher carbohydrate and lower nitrogen content of these plants.

6. The addition of calcium nitrate in very dilute solution to the water in which the seeds were soaked did not prevent the deleterious influence of soaking on germination and growth.

7. Soaking the seeds resulted in a marked relative increase in reducing sugars, starch and total carbohydrates, and solid matter, and in a relative decrease in total nitrogen, organic nitrogen, amino-acid

nitrogen, and proteins in the mature plants produced from the soaked seeds. A correlation may be seen in the decreased nitrogen associated with decreased moisture and increased carbohydrates.

8. It seems probable that the greater relative accumulation of carbohydrates and deficiency of nitrogen compounds in the plants from the soaked seeds are due to a decrease in the ability of these plants to form the nitrogen compounds from the carbohydrates. The reduction in growth is probably due to the reduction in nitrogen compounds.

9. There was an increase in the rate of production of respiratory carbon dioxide in the sprouted seeds soaked before sprouting in comparison with those sprouted without previous soaking. This increase was probably due to the fact that the treated seeds contained considerably more water than untreated ones. The higher water content was favorable to a more rapid rate of respiration. The reduction in growth, of course, was not connected with any decrease in the rate of respiration.

10. Soaking the seeds without aeration resulted in a regular decrease in the catalase activity. Soaking with aeration resulted at first in a small decrease in catalase activity, but this was soon followed by a considerable increase. This modification of the result is evidently due to the fact that the seeds when soaked with aeration pass through the early stages of germination, and consequently there is an increase in catalase activity characteristic of seed germination.

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